Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

The effect of guanidin on plants*

OSWALD SCHREINER AND J. J. SKINNER

INTRODUCTION

Guanidin has been found in some plants. It may arise from the oxidation of arginin. Arginin is a decomposition product of proteins and exists in plants, and it has also been found in soils.† Interesting in this connection is the appearance of guanidin in seedlings, as reported by Schulze,‡ which probably results by further changes from arginin. Guanidin can also be formed by the oxidation of guanin in the laboratory.

Guanidin was shown to be toxic to plants, first by Kawakita § and later in this laboratory.|| According to Shibata,¶ who studied the effect of *Aspergillus niger* on a number of nitrogenous compounds, this mold had no effect on guanidin. Hutchinson and Miller** include guanidin among the nitrogenous compounds assimilated by plants.

In the earlier experiments in this laboratory the guanidin carbonate was studied in various concentrations of distilled water. The wheat plants were killed in 9 days in solutions stronger than 100 parts per million. In all the lower concentrations, including 1 part per million, the wheat plants were seriously injured.

This harmful effect of guanidin has since then been more

---

* Contribution from the Laboratory of Soil Fertility Investigations, Bureau of Soils.

† Schreiner, O., and Shorey, E. C. The presence of arginin and histidin in soils. Jour. Biol. Chem. **8**: 381. 1910.

‡ Schulze, E. Ueber einige stickstoffhaltige Bestandtheile der Keimlinge von *Vicia sativa*. Zeit. Physiol. Chem. **17**: 193. 1893.

§ Kawakita, I. On the behavior of guanidin to plants. Bull. Col. Agr. Tokio **6**: 181. 1904–05.

|| Schreiner, O., Reed, H. S., and Skinner, J. J. Certain organic constituents of soils in relation to soil fertility. U. S. Dept. Agr. Bur. Soils Bull. 47. 1907.

¶ Shibata, K. Über das Vorkommen von Amide spaltenden Enzymen bei Pilzen. Hofmeister's Beiträge **5**: 384. 1904.

** Hutchinson, H. B., and Miller, N. H. J. The direct assimilation of inorganic and organic forms of nitrogen by higher plants. Centralbl. Bakt. **30**: 513. 1911.

thoroughly studied, and in this later work nutrient culture solutions, 66 in number, have been used, comprising all the combinations of potash, phosphate, and nitrate, as explained below.

EXPERIMENTAL METHODS

Wheat seedlings were grown in aqueous culture solutions containing the ordinary fertilizer salts, calcium acid phosphate, sodium nitrate, and potassium sulphate. Some of the cultures contained calcium acid phosphate only, some sodium nitrate only, and some potassium sulphate only. Other solutions were composed of mixtures of two salts, sodium nitrate and calcium acid phosphate, sodium nitrate and potassium sulphate, and calcium acid phosphate and potassium sulphate. Still other solutions had all three constituents in various proportions. The concentration of all the solutions was 80 parts per million of the fertilizer ingredients, P_2O_5, NH_3, and K_2O. In cultures containing only one fertilizer salt, for instance calcium acid phosphate, the concentration was 80 parts per million of P_2O_5. If two salts were present, for instance calcium acid phosphate and sodium nitrate, the concentration was 80 parts per million of $P_2O_5 + NH_3$. If all three salts were present, the concentration was 80 parts per million of $P_2O_5 + NH_3 + K_2O$. The ratios of the constituents varied in 10 per cent stages. In all, there were 66 different cultures of nutrient solutions.

For a more detailed explanation of this triangular scheme and the principles involved in preparing culture solutions in definite progression of its constituents, the reader is referred to earlier publications.*

Two sets of cultures were prepared; to one set was added merely the nutrient salts, while to each culture of a similar set guanidin carbonate was added in amounts of 25 parts per million in addition to the nutrient salts. The culture solutions were contained in wide-mouth bottles, holding 250 c.c., and 10 wheat seedlings grown in each culture. The culture solutions were changed every three days, four changes being made in the course of the experiment. The solutions were analyzed for nitrates immediately after each change. The phosphate and potassium were determined on a composite solution of the four changes. Observations on the general development of the plants and the effect on root growth and appearance were made during the experiment, and photographs were taken.

---

* Schreiner, O., and Skinner, J. J. Some effects of a harmful organic soil constituent. U. S. Dept. Agr. Bur. Soils Bull. 70. 1910. Ratio of phosphate, nitrate and potassium on absorption and growth. Bot. Gaz. **50**: 1. 1910.

For the purpose of preparing the 66 culture solutions needed in this investigation, stock solutions of the three salts, calcium acid phosphate, sodium nitrate, and potassium sulphate, were prepared separately. The salts were chemically pure salts and were dissolved in each case in physiologically pure water. For the calcium acid phosphate solution 1.776 grams of $CaH_4(PO_4)_2.H_2O$ per liter were used. This solution has a concentration of 1,000 parts per million of P_2O_5. The sodium nitrate solution was prepared by using 5,000 grams of $NaNO_3$ per liter. This solution is equivalent to a concentration of 1,000 parts per million of NH_3. The potassium sulphate solution was prepared by dissolving 1.852 grams of K_2SO_4 per liter. This solution has a concentration of 1,000 parts per million of K_2O. The amount of culture solution used in each culture bottle being 250 c.c., it follows that every 2 c.c. of these 1,000 parts per million stock solutions will represent 8 parts per million in the culture solution when this is diluted to the capacity of the bottle; i. e. the successive addition of 2 c.c. of the stock solution gives the 10 per cent differences desired in putting up the 66 solutions. In putting up the 66 cultures it was found desirable to calibrate each bottle for 250 c.c. capacity and to number them consecutively from 1 to 66. Bottles in which the 250 c.c. mark was either high or low were discarded, only those being used that brought the surface of the liquid from one-half to one centimeter from the top. These 66 bottles were then arranged in a triangular form, as illustrated by FIG. 1 and 2.

The necessary amount of the 1,000 parts per million stock solution above described was measured from a burette. For instance, in adding the requisite amount of nitrate to the set of culture bottles the line of bottles P to K in FIG. 2 received no nitrate solution, the next line of bottles received 2 c.c. each, the third line 4 c.c. each, and so on, increasing 2 c.c. with each successive line, the culture at N receiving 20 c.c. of the nitrate solution.

Likewise, in adding the requisite amount of potash, the cultures in the line P to N received none, the cultures in the next line received 2 c.c. each, in the third line 4 c.c. each, and so on up to the culture at K, which received 20 c.c. of the potash solution. The phosphate solution is added in the same manner, none to cultures in line K to N, 2 c.c. to cultures in the second line, and so on up to the culture at P, which received 20 c.c.

Each bottle received, therefore, a total of 20 c.c. of one, two, or three of the stock solutions, depending upon whether it was at the apex, along the sides, or in the interior of the triangle. All of the cultures were then diluted up to the 250 c.c. mark.

In this investigation, as already mentioned, the culture solution contained 25 parts per million of the guanidin carbonate. The amount of this carbonate to be added to each culture bottle of 250 c.c. was, therefore, 6.25 milligrams. Of the 250 c.c., 20 c.c. were already contained in the bottles in the form of the fertilizer salt solution. Therefore the solution with which the fertilizer salt solution in the bottles was to be diluted consisted of 6.25 milligrams of the compound dissolved in 230 c.c. of pure water or 27.17 milligrams per liter. A sufficient quantity of this strength of solution was prepared to fill the 66 bottles.

As a means of comparing the cultures grown in these solutions containing the guanidin, it was necessary to put up cultures prepared in exactly the same way, except that pure water was used.

In all of this work physiologically pure water was used. This was prepared by shaking ordinary distilled water with a highly absorptive carbon black, as described in earlier publications,* which removes from the water any injurious property it may possess.

The culture solutions were now ready to receive the plants, which were thereafter grown in a greenhouse under suitable conditions.

In this work it was necessary to have a large number, often several hundred, and sometimes thousands of uniform seedlings, i. e. seedlings of the same age and equal development and general vitality.

The manner of growing the seedlings and the method of inserting them in the above culture solutions were as follows:

---

* Livingston, B. E., et al. Further studies on the properties of unproductive soils. U. S. Dept. Agr. Bur. Soils Bull. 36. 1907.

Perforated aluminum disks were floated, by means of a raft prepared from sealed glass tubing, in such a way that the disks were kept just at the surface of the water when loaded with seeds.

The wheat seeds, previously soaked in water, for about two hours, not longer, were spread evenly on the surface of the disks. The perforations in the 1.6 mm. thick aluminum were approximately 3.2 mm. in diameter and 2 mm. apart. The entire arrangement of raft and disks was floated in a porcelain-lined iron tank. The seedlings were used when the plumule was about 2 cm. high and just ready to emerge from the enveloping sheath. In this manner the 1,320 uniform seedlings required for each experiment were readily obtainable.

The bottles used in these cultures were made of flint glass and were stoppered by means of a soft flat cork about 12 mm. in thickness and notched for holding the seedlings. The method of notching these corks consisted in cutting 10 vertical, triangular wedges from the circumference of each. Each wedge after being cut out was truncated, so that when it was replaced, a small triangular opening was left through which the plumule of the seedling passed. This hole was large enough to hold the seedling firmly and yet not bruise or injure it in any way by pressure. Around the circumference of the cork, in the upper half, a groove had been made sufficiently large to hold a small rubber band. After the wedges were inserted, the band kept them in place and allowed the cork with the seedlings to be handled readily and put into or taken out of the bottle without disturbing the plants.

As already mentioned, these solutions were changed every three days. This was done by putting up other triangles of bottles similar in every respect to the ones just described. The corks with the plants were then transferred from the old solution to the corresponding new solution.

EFFECT OF GUANIDIN ON WHEAT

As already mentioned, two sets of the cultures were prepared; one was used as a control; to the other was added guanidin carbonate, 25 parts per million to each culture. The wheat seedlings grew from February 15 to February 27, 1911, the solutions being changed every three days.

For the first few days no difference was noticeable between the control and the guanidin set. About the fifth day bleached spots appeared in the leaves in some of the cultures, producing an effect like a plant disease. The diseased spots on the leaves spread and became larger and more numerous. The spots appeared first on a few cultures, but when the cultures were arranged in the order of their composition the reason for this became evident. The guanidin effect showed itself first in the cultures high in nitrate and then spread to those lower in nitrate, until the series of no nitrate content was reached. In this series the guanidin effect was scarcely discernible. As the plants grew older, the guanidin effect became more and more marked; the bleached spots coalesced and appeared most marked in the lower part of the leaves; the leaves broke finally, presenting on the whole an effect similar to that produced by a wilt disease and accompanied with considerable bleaching of the green parts of the plant. This, at least, was the effect in all of the cultures containing nitrate and was the more marked the higher the nitrate content. The series containing no nitrate whatever retained its green color and was not subject to collapse or wilting, although here and there some bleached spots appeared.

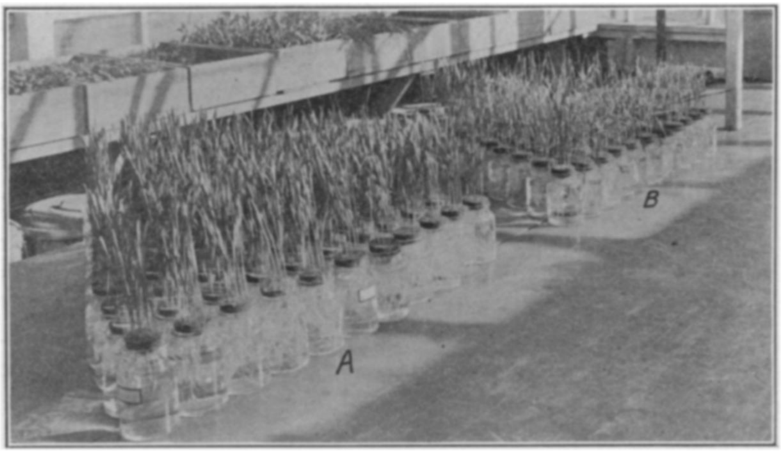

FIG. 1. Effect of guanidin on wheat plants; series *A* without guanidin, series *B* with guanidin.

In FIG. 1 are shown the two sets of cultures. *A* is the normal or control set and *B* the guanidin set. The distinctly destructive effect of the guanidin is apparent in the blighted appearance of set *B* in striking contrast to the fine growth in the control cultures, which is even more marked than the photograph can show because of the deep green color of the normal set contrasted with the bleached appearance of the guanidin set.

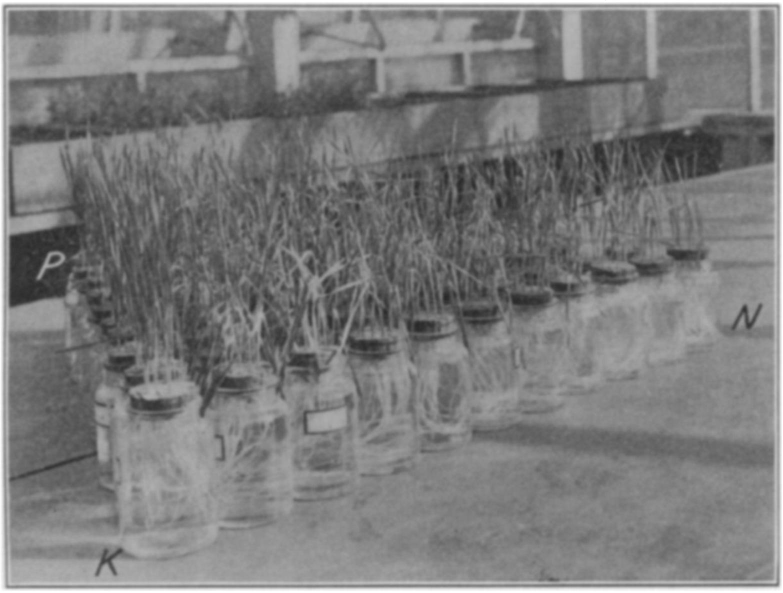

FIG. 2. Effect of guanidin on wheat plants. Nearer view of series *B* in FIG. 1.

In FIG. 2 a nearer and better view is had of this guanidin set. The plants are arranged according to the composition of their culture solutions, thus forming a triangle. By this arrangement the highest nitrate culture appears at *N*, the highest phosphate culture at *P*, and the highest potash culture at *K*. It is apparent that the poorest plant development occurs in those cultures high in nitrate but is distinctly noticeable in every culture except in the line of cultures from *P* to *K*, which is the series containing no nitrate. The plants in this line of cultures are firm and erect, as can be seen in the photograph. In actuality this difference is accentuated by the nearly normal green color of this series, whereas all other cultures, in addition to the dilapidated condition, had a decidedly bleached appearance.

The effect of the nitrate in increasing the harmful effect of guanidin is also shown by the weight of the tops taken at the termination of the experiment. In TABLE I the first column gives the series, the cultures of which have like nitrate content. The amounts of nitrate in each culture of these series are given in the second column. The third and fourth columns give the weight of the tops of the plants grown in the cultures without and with 25 parts per million of guanidin carbonate. It is at once apparent that the guanidin is very harmful to growth. The total green weight of the 66 cultures in the normal set was 151.2 grams against only 99.5 grams in the guanidin set. Placing the normal at 100, the growth in the guanidin set becomes 66. The last column gives the relative growth in each of the series of uniform nitrate content in the set. The culture that contained 80 parts per million NH_3 as nitrate gave a relative weight of only 31 or a decrease in growth of 69 per cent, whereas in the series of cultures that contained no nitrate the relative growth was 94, a decrease of only 6 per cent below the control. As shown in the table, the relative weight of tops obtained increased with decreasing nitrate content.

TABLE I

EFFECT OF GUANIDIN ON GROWTH AS INFLUENCED BY NITRATE

Culture series	NH_3 as nitrate in fertilizer mixture, parts per million	Green weight of cultures, grams		Relative growth. Control = 100
		Without guanidin	With 25 parts per million guanidin	
66	80	1.656	0.520	31
55–65	72	4.178	1.990	47
45–64	64	6.860	3.412	48
36–63	56	9.449	5.031	53
28–62	48	12.359	6.874	55
21–61	40	15.998	8.394	52
15–60	32	18.649	11.491	62
10–59	24	21.691	13.602	62
6–58	16	23.327	15.709	67
3–57	8	20.923	17.288	83
1–56	0	16.156	15.195	94

The effect of guanidin on growth, and especially the harmful influence of the nitrate, was considered so remarkable, particularly in the light of the previously observed beneficial effect of nitrate in conjunction with harmful compounds,* that the entire experiment was repeated, and it gave absolutely the same results.

The plants grew from March 17 to March 29. Again the effect of the guanidin did not appear until about five days had elapsed, and again it showed itself first, and later most marked, in the high nitrate solutions. Again the no nitrate cultures were left practically unharmed. The total green weight for the normal set was 163.9 grams, and in the guanidin set it was 116.3 grams, a relative green weight of 71. The weights of the tops in the different nitrate series are given in TABLE II, the arrangement being exactly the same as in TABLE I. The last column, giving the relative green weight of the different nitrate series, again shows the greater harmfulness of the guanidin in the series containing nitrate, which is especially marked in those high in nitrate.

TABLE II

EFFECT OF GUANIDIN ON GROWTH AS INFLUENCED BY NITRATE

Culture series	NH_3 as nitrate in fertilizer mixture, parts per million	Green weight of cultures, grams		Relative growth. Control = 100
		Without guanidin	With guanidin	
66	80	2.201	1.003	46
55–65	72	4.040	2.040	50
45–64	64	7.275	4.021	55
36–63	56	10.359	5.996	57
28–62	48	13.387	7.544	56
21–61	40	17.394	10.110	58
15–60	32	20.821	13.204	63
10–59	24	22.769	16.952	74
6–58	16	25.614	18.141	71
3–57	8	24.097	21.112	81
1–56	0	15.969	16.164	100

In the first experiment the solutions were also analyzed for phosphate, nitrate, and potash. The results show a marked decrease in the absorption of the nutrient salts on the part of the guanidin plants, although the roots themselves were not noticeably affected, as is apparent from the plants visible in FIG. 1 and 2.

The total phosphate, nitrate, and potash removed by the normal plants was 1,608.9 milligrams, against only 1,088.5 milligrams in the guanidin set. The phosphate removed was 427.3 milligrams in the control and 287.0 milligrams in the guanidin set; the potash was 723.7 milligrams for the control and 496.7 milligrams for the guanidin set; the nitrate was 457.9 milligrams for the control cultures and 304.8 milligrams for the guanidin cultures.

---

* Schreiner, O., and Reed, H. S. The power of sodium nitrate and calcium carbonate to decrease toxicity in conjunction with plants growing in solution cultures. Jour. Amer. Chem. Soc. **30**: 185. 1908.—Schreiner, O., and Skinner, J. J. Some effects of a harmful organic soil constituent. Bot. Gaz. **50**: 161. 1910.

Tests of guanidin carbonate in quantities of 50 parts per million were also made in soil cultures. The physiological effect of the guanidin was again apparent in the spotting and bleaching of the leaves and was again most evident in the soils fertilized with nitrate.

EFFECT OF GUANIDIN ON OTHER PLANTS

The effect of guanidin was further studied by using plants other than wheat. In FIG. 3 is shown its effect on corn growing in culture solution no. 41, containing 16 parts per million phosphate, 32 parts per million nitrate, and 32 parts per million potash. FIG. 4 shows the effect of guanidin on cowpeas, the control and guanidin cultures being in duplicate. FIG. 5 shows its effect on the potato plant. In these tests guanidin was used in concentra-

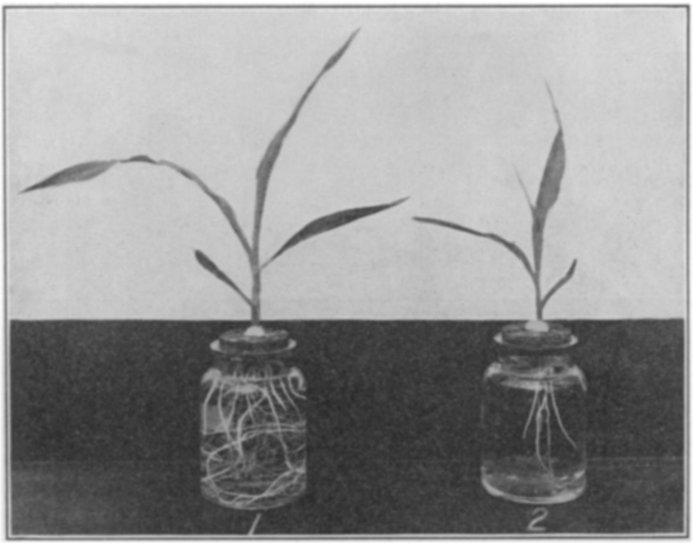

FIG. 3. Harmful effect of guanidin on corn; no. 1 without guanidin, no. 2 with guanidin.

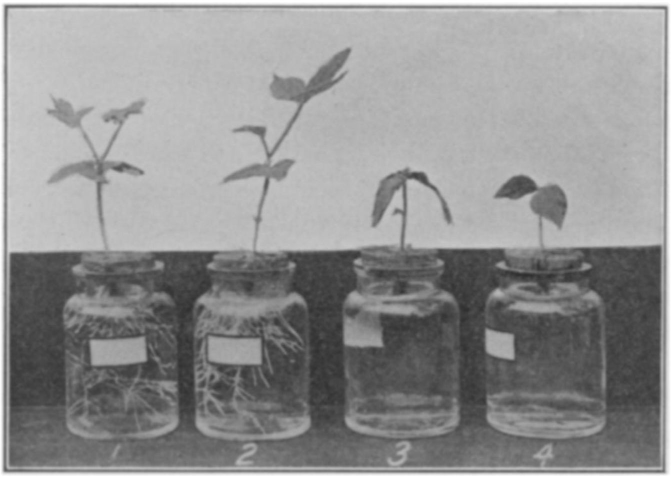

FIG. 4. Harmful effect of guanidin on cowpeas; no. 1 and 2 without guanidin, no. 3 and 4 with guanidin.

tions of 25 parts per million. With all these plants the harmful effect on growth is clearly shown and the same general physiological action was manifested as in the case of wheat.

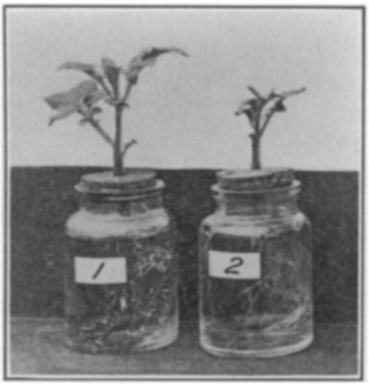

FIG. 5. Harmful effect of guanidin on potatoes; no. 1 without guanidin, no. 2 with guanidin.

INFLUENCE OF ORGANIC NITROGENOUS COMPOUNDS

In regard to the influence of nitrate in increasing the harmfulness of guanidin some further studies were made which indicate that other nitrogenous compounds do not share this property with the nitrate.

To solution no. 41, containing 16 parts per million phosphate, 32 parts per million nitrate, and 32 parts per million potash, 25 parts per million of guanidin were added, and wheat seedlings were grown in this solution as well as in a control without guanidin. A similar set of cultures was prepared with and without guanidin, but with this difference that the nitrate was omitted entirely and an equivalent amount of nitrogen in the form of asparagin was added to the culture solution. The plants grew from March 10 to March 23, 1911.

The characteristic effects of guanidin were noticed in the culture containing nitrate but did not appear in the culture containing asparagin. The appearance of the cultures is shown in FIG. 6, where no. 1 is the control with nitrate and no. 3 the control with asparagin. No. 2 and 4 are the guanidin cultures to be compared with each other and with their respective controls. No. 2 shows the wilting effect of the guanidin as well as the decreased growth. The colors of the various cultures brought out the difference even more strikingly. The green weights given in TABLE III bear out the same point.

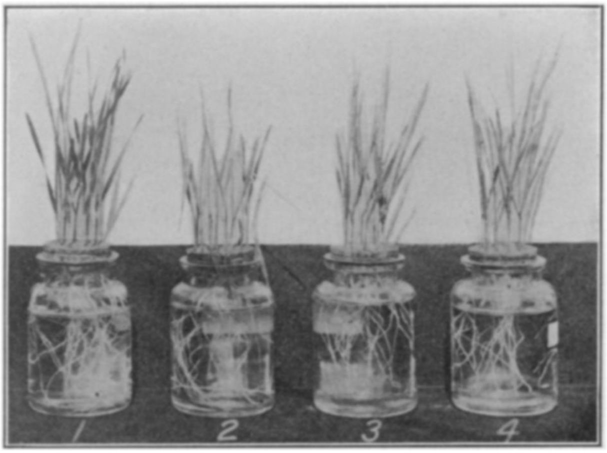

FIG. 6. Effect of guanidin with nitrogen in different forms; no. 1, nutrient solution containing sodium nitrate; no. 2, same plus guanidin; no. 3, nutrient solution containing asparagin; no. 4, same plus guanidin.

TABLE III

EFFECT OF GUANIDIN IN CULTURE SOLUTIONS CONTAINING NITROGEN AS NITRATE AND AS ASPARAGIN

Culture solution	Green weight, grams
$CaH_4(PO_4)_2$ $NaNO_3$ K_2SO_4	4.0
$CaH_4(PO_4)_2$ $NaNO_3$ + 25 parts per million guanidin K_2SO_4	2.5
$CaH_4(PO_4)_2$ Asparagin K_2SO_4	3.5
$CaH_4(PO_4)_2$ Asparagin + 25 parts per million guanidin K_2SO_4	3.3

Creatinin was also tried in this same manner. Creatinin was identified as a soil constituent in this laboratory[*] and also found to be a constituent in a number of plants.[†] Its effect on plants has been shown to be beneficial, its action being to replace nitrate in producing plant growth.[‡] The results obtained by using creatinin as the source of nitrogen instead of sodium nitrate in conjunction with the guanidin are given in TABLE IV.

TABLE IV

EFFECT OF GUANIDIN IN CULTURES CONTAINING NITROGEN AS NITRATE AND AS CREATININ

Culture solution	Green weight, grams
$CaH_4(PO_4)_2$ $NaNO_3$ K_2SO_4	3.4
$CaH_4(PO_4)_2$ $NaNO_3$ + 25 parts per million guanidin K_2SO_4	2.4
$CaH_4(PO_4)_2$ Creatinin K_2SO_4	3.0
$CaH_4(PO_4)_2$ Creatinin + 25 parts per million guanidin K_2SO_4	3.0

---

[*] Shorey, E. C. The isolation of creatinin from soils. Jour. Amer. Chem. Soc. **34**: 99. 1912.

[†] Sullivan, M. X. The origin of creatinin in soils. Jour. Amer. Chem. Soc. **33**: 2035. 1911.

[‡] Skinner, J. J. The beneficial effect of creatinin and creatin on growth. Bot. Gaz. **54**: 152–163. *f. 1.* 16 Au 1912.

Again the characteristic effect of the guanidin was observed in the cultures containing nitrate, and the depressed growth is shown by the green weight in the table. With creatinin the effect of guanidin was not apparent either in appearance or in the green weight obtained.

SUMMARY

Guanidin, as carbonate, is shown to be harmful to wheat, corn, cowpeas, and potato plants. It produces an effect similar to a physiological disease. The plant is normal for a few days, then begins to show a spotted appearance on leaf and stem. This effect develops until the plant is bleached to a considerable extent, with final collapse.

This harmful effect of guanidin on plants is augmented by the presence of nitrate and increases with the amount of nitrate present.

Sources of nitrogen, other than nitrate, did not show this same effect. The organic nitrogenous compound asparagin, as well as the beneficial soil constituent creatinin, appeared in fact to be able to counteract the effect of guanidin itself.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS,
WASHINGTON, D. C.